Dissonant Synapses Shall Be Punished

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http://dx.doi.org/10.1016/j.neuron.2015.06.040

Synaptic activity in the neonatal brain is often locally synchronized. How such clustering comes about is unclear. Winnubst et al. (2015) show that the refinement of synaptic connectivity is driven by the depression of synapses that are asynchronous with their neighbors.

In the neonatal cortex, well before sensory information starts to drive synaptic input, neurons display patterns of spontaneous activity, which is thought to sculpt the emerging synaptic network in an experience-independent way. It has been proposed that spontaneous activity serves as a self-organizing and perhaps Hebbian mechanism to strengthen "relevant" synaptic connections (Khazipov and Luhmann, 2006). Each neuron receives synaptic inputs from a plethora of afferents, resulting in a "salt and pepper"-like connectivity, with neighboring inputs on a single dendrite likely being of different origins (Grienberger et al., 2015). Nonetheless, neighboring synapses on the same dendrite are often found to display more synchronous spontaneous activity as compared to synapses that are located farther away, suggesting that inputs with similar functional features converge on single dendritic loci (Kleindienst et al., 2011; Takahashi et al., 2012). The emergence of such synaptic clusters predicts that during development there is interplay between similarly tuned neighboring synapses, which promotes their stabilization. In this issue of *Neuron*, Winnubst et al. (2015) tested this hypothesis and explored the causal relationship between the coactivity of nearby synapses and the stability of their activity rates.

The authors first investigated in the neonatal mouse visual cortex in vivo whether spontaneous activity of neighboring synapses is correlated. To this end they expressed the genetically encoded calcium indicator GCaMP6 together with the cytosolic marker DsRed in L2/3 pyramidal neurons. The calcium responses of putative synaptic loci on dendrites were imaged, while at the same time, using a patch pipette, synaptic currents were recorded at the soma. Using this approach the authors were able to locate and chronically measure the activity of single synapses. As predicted by previous work (Kleindienst et al., 2011; Takahashi et al., 2012), spontaneous activity of single synapses that were located within short distances (< 12 μm) of one another on the same dendrite were more often coactive than synapses at greater distances. However, they also noticed that those nearby synapses that were coactive for less than 20% of the time often started to display reduced activity rates within minutes after the asynchronous events (Figure 1). On the other hand, if synapses within the same distance were synchronously active, their activity rates did not diminish. For synapses at larger distances, it did not matter if they were coactive or not, as they maintained their activity rates unaltered irrespective of the degree of synchronicity. Interestingly, the temporal window over which asynchronous activity was most effective in causing depression was comparable to the 1–2 s duration of spontaneous activity bursts that have been observed in the retina and visual cortex (Ackman et al., 2012). Although the authors did not investigate in detail the fate of the depressed synapses, they are likely candidates for pruning from the synaptic circuit.

To investigate the causal relationship between asynchronous activity of synaptic neighbors and the loss of their activity rates, the authors moved to an organotypic slice culture of the hippocampus. They first confirmed that the correlation between neighboring synapse maintenance and synchronicity was not restricted to visual cortex. Reassuringly, here, too, synapses within the same short distances that were not activated synchronously were more likely to become depressed. The temporal window again matched the typical burst duration that is normally observed in hippocampus (~400 ms). In order to interfere with the synchronicity between neighboring synapses, they performed a minimal and low-frequency stimulation of presumptive afferents. Such microstimuli desynchronized the activity of individual synapses relative to their spontaneously active neighbors. This out-of-sync activity increased the failure rate of subsequent evoked activity, an effect that lasted for at least 50 min. Although this strongly suggests that the asynchrony underlies increased synaptic failure, the low-frequency stimulus itself could have caused the depression.

Thus, to gain even better control over the synchronicity between synapses, and to produce activation patterns similar to those of spontaneously active synapses, they designed a closed-loop stimulation paradigm. As before, they selected single synapses whose activity was precisely controllable with an electrical microstimulus. The stimulus was then linked to the automatic detection of spontaneous activity (i.e., calcium responses) of local or distant synapses. Whereas co-stimulation with local synapses left the stimulus-evoked activity unaffected, the co-stimulation with distant synapses increased their failure rates. As the synchronization with distant synapses indirectly caused asynchrony with neighbors, the authors conclude that the out-of-sync activity provides an active signal for synaptic depression. Altogether, the authors' experiments point to

a mechanism in which clustering is driven by synaptic depression due to asynchrony rather than by potentiation due to synchrony.

What are the underlying mechanisms that drive this depression? Since the change in synaptic activity rates was not accompanied by a decrease in the amplitudes of those events, the authors inferred that the drop in frequencies was the result of reduced presynaptic transmitter release probabilities. They investigated how asynchronous activity at the postsynapse could influence the failure of presynaptic release. Based on the literature, the authors speculated that proBDNF/p75^{NTR} signaling was a likely retrograde messenger to underlie this process. Indeed, a bath-applied antagonist of p75^{NTR} prevented the increase in failure rates upon low-frequency microstimulation. On the other hand, application of noncleavable proBDNF depressed the activity rates of synapses that were initially highly coactive and prevented the clustering of coactivity over time. The authors propose that proBDNF/p75^{NTR} signaling may act as a local mediator to "punish" out-of-sync synapses (Figure 1). Other factors are likely to be involved in determining the distance over which this synaptic interaction occurs (Govindarajan et al., 2006).

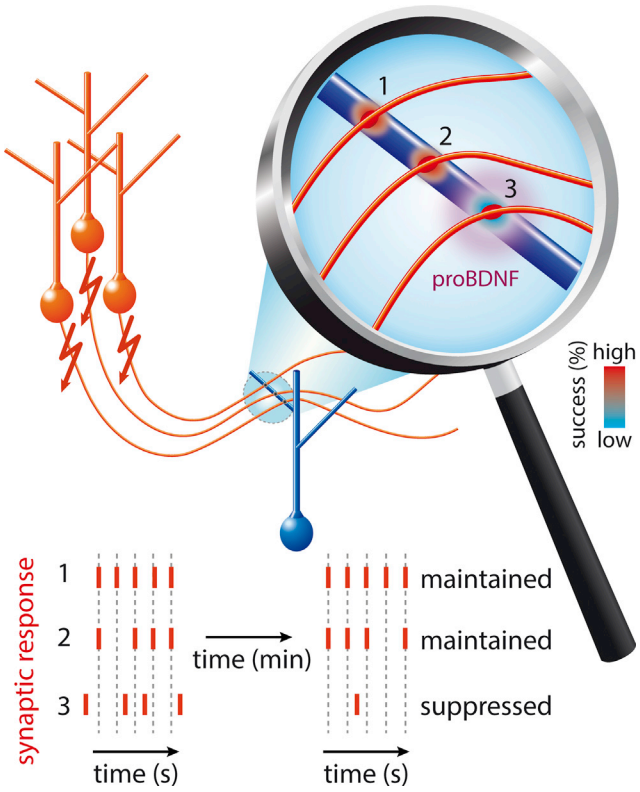

Figure 1. Depression of Synapses that Are Asynchronous with Their Neighbors

Schematic of three spontaneously active neurons (red) that bear synaptic connections with the same target neuron (blue). The synapses of neurons 1 and 2 are activated synchronously over a timescale of seconds, but they are out of sync with synapse 3. Within minutes after the asynchronous events, the success rate for responses of synapse 3 becomes suppressed. Only synapses that are asynchronous with their neighbors located within ~12 μm are depressed. The activity rates of synapses 1 and 2 remain unaltered. The depression effect is presynaptic and locally mediated by proBDNF/p75^{NTR} signaling.

The finding by Winnubst et al. (2015) will influence our thinking about synaptic circuit development. Synaptic inputs from hippocampal CA3 neurons that share a developmental time window have been found to anatomically cluster on CA1 dendrites (Druckmann et al., 2014). Together with the data from Winnubst et al. (2015), this invites the speculation that the characteristic bursts of synchronized activity in groups of hippocampal neurons become a selective force for this observed anatomical clustering and prevent nonsynchronous synapses from intermingling (Kleindienst et al., 2011; Takahashi et al., 2012). The authors' findings also imply that retinal waves (Ackman et al., 2012) may drive the functional clustering of synaptic inputs in accordance with the activity patterns of upstream neurons. It would be interesting in future experiments to test if such a spatiotemporal relationship exists.

The clustering of synapses may have strong implications for the functioning of synaptic networks. Coactivity of nearby-located synapses may elicit nonlinear dendritic responses (Major et al., 2013), which have been predicted to increase the information processing capacity of neurons (Poirazi and Mel, 2001). However, as yet the spatiotemporal prerequisites for nonlinear synaptic integration in vivo have not been fully explored.

Synaptic clustering also impacts neuronal plasticity and learning. Sensory experience (Makino and Malinow, 2011) or motor learning (Fu et al., 2012) has been shown to drive plasticity in nearby synapses. Thus, synaptic clustering may affect, and even be the result of, a generalized and continuous interplay between nearby postsynaptic structures (Harvey and Svoboda, 2007; Oh et al., 2015).

The question remains whether the observed clustering of spontaneous activity will be relevant for synaptic circuits underlying behavior and sensory processing. Nearby synapses that were sculpted by spontaneous activity may not necessarily continue to share response patterns during behavior. Indeed, synaptic activity as induced by simple visual stimuli can be found dispersed over the dendritic tree (Grienberger et al., 2015). However, this does not exclude the possibility that inputs conveying complex and higher-order information may locally converge on dendrites together with synapses bearing low-order information. Furthermore, dendritic nonlinear events may drive plasticity (Gambino et al., 2014). Thus, it is conceivable that spontaneously clustered synapses may heterosynaptically strengthen functionally relevant, "salt and pepper"-like, synaptic inputs over larger stretches of dendrites through the generation of such events.

In summary, the work by Winnubst et al. (2015) now firmly establishes that the degree of local synchronicity between synapses determines the stability of their activity. Synapses that are not spontaneously coactive with their neighbors will be punished. Thus, contrary to what one may have expected, synaptic clustering likely results from the depression of asynchronous synapses. This functional clustering may set the stage for behaviorally relevant synaptic circuits later in life in which nonlinear interactions between

nearby synapses will have an important role to play.

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Parvalbumin Interneurons: All Forest, No Trees

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http://dx.doi.org/10.1016/j.neuron.2015.06.041

There has been a surge of interest in how inhibitory neurons influence the output of local circuits in the brain. In this issue of *Neuron*, Scholl et al. (2015) provide a compelling argument for what one class of inhibitory neurons actually does.

What is cortical inhibition good for? Recently, the answer to this question is remarkably similar to one of those questions on "Family Feud," where there's a survey of opinions and the top 10 answers are all correct. Fortunately, the results from Scholl et al. (2015) in this issue of *Neuron* add enough new data to tip the scales in favor of one simple answer.

In the neocortex, inhibitory neurons are a fairly small minority, comprising roughly 20% of all cortical neurons. Historically, this has made it difficult to find these cells and to record from them in the intact brain. Even more maddening, this small population is subdivided, very roughly, into three groups (and more likely a dozen), based on their interaction with excitatory neurons (Kawaguchi and Kubota, 1997). Parvalbumin (PV)-expressing interneurons fire rapid barrages of action potentials, and are accordingly named "fast-spiking" interneurons. These innervate and inhibit the cell bodies of excitatory neurons. Somatostatin (SOM)-expressing interneurons have firing rates that are more on par with the local excitatory neurons, and are thus often referred to as "regular-spiking." These innervate and inhibit the dendrites of pyramidal neurons. In the primary visual cortex, where these cells have been most extensively studied, both groups receive strong excitatory input. The final group of inhibitory neurons is characterized by their expression of vasoactive intestinal polypeptide (VIP). These cells appear to inhibit other inhibitory neurons and to receive neuromodulatory input from the brainstem, and are thought to regulate brain states during arousal (Hangya et al., 2014; Pfeffer et al., 2013).

Over the past half-decade or so, a number of mouse lines have been developed in which expression of the gene encoding the bacteriophage tyrosine recombinase enzyme, Cre recombinase, is directed by PV, SOM, or VIP promoter/enhancer elements (Pfeffer et al., 2013). These mice have given us the ability to finally visualize and manipulate each inhibitory class.

Scholl et al. (2015) provide new data supporting a view that the computational heavy lifting in the cortex is done by the excitatory neurons, whereas PV cells seem to leave a lot of potentially very useful information on the table. Rather than integrating specific cortical inputs to create complex receptive fields that extract higher-order information from the visual scene, as excitatory neurons do (Cossell et al., 2015), PV cells simply integrate inputs from the local network without specificity (Figure 1). Being uniformly connected to the local excitatory neurons makes PV cells well suited to a different role—monitoring and regulating the total activity of the local network, also known as gain control.

To reach this conclusion, they imaged the activity of large numbers of excitatory and PV neurons in mouse primary visual cortex using two-photon excitation of the calcium indicator Oregon Green